On the 7-azaindole in acetonitrile anhydrous solutions as an inapropriate photophysical model for DNA base pairs.

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Multiple Hydrogen-bonding is a fundamental issue to explain base-pairing in DNA structures, which was firstly described by Watson and Crick[1] using stable keto tautomer forms. In their analysis, they considered the possibility of mutations via proton transfer reactions within a base pair. Such reactions can be induced by electronic excitation, for example, adenine-cytosine mispairing may be caused by double-proton phototautomerism [2]. The 7-azaindole (7AI) doubly-hydrogen bonded dimer was firstly proposed as a suitable model for explaining the DNA base mispairing owing to excited state two-proton phototautomerization in 1969 by Taylor et *al.*[3]. The concerted nature of this excited state biprotonic transfer has been strongly supported by available evidence (see references 4-6 and references therein). Recently, Kwon and Zewail (K&Z) [7] have reported femtosec time-resolved evidence on the stepwise mechanism in polar solvents, using very

concentrated solutions of 7AI (0.1 M) in anhydrous acetonitrile, diethylether and dichloromethane on excitation at 320 nm. However, based on a careful spectroscopic analysis of the absorption and emission spectra of anhydrous 0.1 M 7-azaindole solutions in acetonitrile and butyronitrile, we demonstrate in this letter that the 7AI molecule does not form the doubly hydrogen bonded dimer at room temperature (rt) in acetonitrile, but it does generate another aggregate which emits fluorescence at ca. 500 nm. Consequently, the assertion of Kwon and Zewail [7,8] that the rate of proton transfer in 7-azaindole dimers is significantly dependent on the solvent polarity and its stepwise mechanism for the process is not rightly stated as no C_{2h} dimer is formed in the medium used to record their femtosecond time resolved and fluorescence spectroscopic evidence (viz. 7-azaindole 0.1 M solution in acetonitrile at rt

The existence of doubly-hydrogen bonded 7AI dimers under those experimental conditions set by K&Z [7] does not agree with some evidence found by our laboratory using 7AI 10^{-4} M in (spectrometric quality) acetonitrile and butyronitrile solutions, and lowering the temperature allows us to conclude that the 7AI monomer does not form C_{2h} dimers; a brief summary of them was published in PNAS as a short letter[9], and in Nature Preceeding[10] : i) absorption, emission, and excitation spectra of 7AI 0.1 M at room temperature and lower temperatures demonstrate that the C_{2h} 7AI dimer is not formed in (spectrometric quality) acetonitrile, diethyl ether, and dichloromethane; and ii) on excitation at 320 nm no emission is found that could be assigned to the double-proton transfer fluorescence in any of the above-mentioned solvents. Therefore, we concluded[9,10] that if there is not 7AI dimer under the conditions set by K&Z, the

excited state double proton transfer must be still regarded as concerted. These authors questioned our results with unfair severity in a much longer reply-letter in PNAS[8] and raised three essential comments, challenging us to reply them, from which the following questions may be inferred: i) if there is no 7AI dimer in anhydrous acetonitrile, why are they able to extract a dimer formation constant?; ii) why are the excitation spectra important under that set of conditions, for instance, at such large concentration?; iii) what are those kinetical changes measured with polarity and with isotopical substitution that their C_{2h} 7AI dimer sample undergoes? This last question is out of context if we demonstrate that for 7AI 0.1 M solution in acetonitrile at room temperature the C_{2h} 7AI dimer is not formed. Indeed, the only reason to assume that the clear display for a buildup of an emission band around 500 nm in polar solvents is that "it is widely accepted as a signature of excited-state double-proton transfer in 7AI dimers"[8]. Finally, they challenged us to demonstrate that the first absorption band for the 7AI 0.1 M solution in polar solvents can be isolated in separate bands for the 7AI monomer and the doubly-hydrogen bonded C_{2h} 7AI dimer.

This letter explores in detail the photophysical behavior of 7AI in acetonitrile under the same anhydrous conditions as those used by K&Z[7], using anhydrous solvent from the beginning and by checking humidity pollution of the samples during the spectroscopic analyses. Also, this work sheds light to the topic of 7AI in polar solvents, and emphasizes the importance of using anhydrous solvents as well as spectrometric

solvents for comparison, and the use of fluorescence excitation spectra for analytic assignments.

Scheme 1

7-Azaindole H-bonded dimer results from the double hydrogen-bonding interaction between the corresponding pyrrole and pyridine units in two 7AI monomers, and is usually referred to as the C_{2h} dimer, see Scheme 1. Structurally, the C_{2h} dimer is the most stable among the five possible 7AI dimers examined to date[11]. Ingham and El-Bayoumi[12] estimated from experimental evidence in 3MP that the dimer-formation constant for 7AI increases from 1800 M^{-1} at 295 K to 10^6 M^{-1} at 200 K. This estimation conforms to the corresponding theoretical calculations[11] that predict an increase of several orders of magnitude for the dimerization constant as the temperature is lowered. This behavior is very important as it can be used for monitoring the appearance of dimer species for a 7AI sample solution by increasing the equilibrium constant as the temperature is lowered. This procedure has been useful to monitor the dimer-formation of 7AI solutions (10^{-4} M) in 3MP[13], 2MB[14], ClBut[15], decalin[16] and and heptane (Figure 8 of Supporting Information). Therefore, the procedure used in this letter to detect dimerization for 7AI in acetonitrile and butyronitrile solutions involve a very slow-down rate for the temperature (about 0.1 K each 12 sec) in order to maintain the solution in near-equilibrium conditions throughout. The absorption and emission spectra obtained at each temperature were re-recorded 5 min later in order to confirm the absence of changes.

Table 1

As can be seen in Table 1, the absorption wavelength maximum of the dimer species is located at 292.2±1.3 nm, and for the monomer species at 287±0.3 nm, while the double-proton-transferred dimer-emission band appears at ca. 479±2 nm. It is important to emphasize the absence of solvatochromic effects for the dimer emission and absorption maxima, which is explained on one hand owing to the zero sensitivity of

the C_{2h} 7AI dimer to solvent polarizability. That is, no solvatochromic change is recorded either for the first absorption band of the normal dimer or for the emission band of the double-proton transferred dimer species as the temperature is varied in the solvents studied. Also, the 7AI dimer exhibits zero dipole moment, and it is neither acid nor basic -the interactions of acids with the π-system of the 7AI dimer can be regarded as negligible.

To demonstrate that a dimerization process is being undergone for 7AI is easy since the first absorption bands for the monomer and dimer exhibit clear-cut differences. For instance, both absorption bands are vibrationally resolved, and that of the dimer is red shifted compared with that of the monomer for about 5 nm —Table 1 (also Figures 9 and 10 of Supporting Information). The first absorption-band of the monomer shows two peak maxima at 287.5±1 nm and at 294.5±1 nm, while the corresponding peaks for the the C_{2h} dimer are located at 293±1 nm and at 299±1 nm[13-16] (see also Supporting Information). It is important to point out that the emission assigned to the two-proton transferred tautomer of the C_{2h} 7AI dimer, independently of the solvent and temperature employed, exhibits a peak maximum at ca. 479 nm and two shoulders at about 427 and 455 nm[13-17].

Three pieces of evidence must be regarded as fundamental to the results obtained in this work on the emission of 7AI in acetonitrile. i) In 1991 Moog and Maroncelli[18] reported the emission for 7AI $3x10^{-5}$ M in acetonitrile solution with the following comments: “The acetonitrile spectrum shows only a single emission band. Although the band has an extended long-wavelength tail, there is no indication of an excited-state proton-transfer reaction in this polar aprotic solvent. This interpretation is supported by the observation of time-resolved emission profiles which are independent of monitoring wavelength”. This long-wavelength tail is monitored up to 540 nm. ii) In 2007, K&Z[7] showed that an anhydrous solution of 7AI 0.1 M in acetonitrile on excitation at 320 nm exhibits a weak emission band without vibronic structure and centred at 500 nm, which

the authors assigned to the C_{2h} dimer of 7AI, and their conclusions are drawn based on the dimer species. iii) In 2008, we showed[10] that an Uvasol grade (from Merck, 99.9% in purity, water content less than 0.01%, from a bottle not recently open) solution of 7AI 0.1 M in acetonitrile on excitation at 320 nm does not show that emission reported by K&Z.

Figure 1

The emission spectra obtained between 298 and 218 K for a 7AI 0.1 M solution in acetonitrile on excitation at 320±1 nm and at 334±4 nm are shown in Figures 1 and 2a, respectively. They comprise two structureless emission bands, one of them of a larger intensity centred at about 350 nm, and another band at about 500 nm. However, on excitation at 290±1 nm the emission spectrum only exhibits the emission centred at 350 nm, Figure 2b. Based on the excitation spectra recorded by monitoring emission light at 385±1 nm, Figure 3, the emission centred at 350 nm is assigned to the monomer species of 7AI in acetonitrile, because of the peaks placed at 294 and 287 nm, which are characteristic of the 7AI monomer species. It is interesting to point out that unexpectively these excitation spectra present an onset at as long wavelength as 330 nm. However, by monitoring emission light at 500±8 nm the excitation spectra, Figure 3, overlaps the first absorption band of the 7AI 0.1 M solution in acetonitrile, and possesses an onset at longer wavelengths, ca. 340 nm, which coincides with that of the first absorption band. These latter spectra exhibit a peak wavelength at 314 nm at 298 K, which shifts to the red as the temperature decreases, i.e. at 218 K it is located at 318 nm. Therefore, the long-wavelength fluorescence is sensitive to solvent polarizability, and must come from some 7AI species which is neither monomer nor the C_{2h} dimer.

Figure 2

At first sight, these spectra obtained by monitoring at 500±8 nm exhibits identical spectral envelop to those by monitoring at 560±8 nm, and must be assigned to another aggregate species of 7AI, which might be in a minor contribution from its low

absorption and emission intensity. This new aggregate species cannot be the C_{2h} dimer because of the sensitivity exhibited to solvent polarizability as the temperature is lowered, and possesses a very different spectral envelop to that of the first absorption band of the compound. It is important to mention that these excitation spectra for the long-wavelength emission slightly increase their intensity as the temperature decreases. The spectral characteristics of this long-wavelength emission band at ca. 500 nm do no conform to those of the C_{2h} dimer, that is, it does not possess vibronic structure, and it is shifted significantly to the red of the C_{2h} dimer emission, which appears at ca. 475 nm, Table 1.

Figure 3

The spectra plotted in Figures 1-3 allow us to conclude that a 7AI 0.1 M solution in acetonitrile does not contain any dimer species at room temperature not even by lowering the temperature down to 218 K. On going from 298 K to 218 K a volume contraction is produced[19], about 11%; it is clear that in order to record the dimer formation vs. the monomer solvation a greater decrease of temperature is necessary or a significant increase of the concentration. A much greater increase of 7AI concentration does not seem to generate the doubly-hydrogen bonded dimer at room temperature (rt) to be detected spectroscopically in acetonitrile; and a greater decrease of temperature than 218 K is not feasible, for the reason that the melting point for acetonitrile is at 225 K, and its optical transparency gets lost at the wavelengths of interest as a result of cracking. Therefore, the only choice for a 7AI 0.1 M solution in acetonitrile at rt is to combine an increase of concentration, by taking advantage of the volume contraction and a slow decrease of the temperature down to the lowest value until the acetonitrile starts crystallization and the sample solution solidifies and cracks. As crystallization will start from the walls to the centre of the cell, it is feasible to reach a final temperature lower than 218 K, and due to volume contraction, at a concentration significantly greater than 0.1 M. As can be seen in Figure 1 in red, a new emission band

is recorded at 202 K and centred at 475 nm, whose spectral envelop exhibits two shoulders at 428 and 455 nm, and that at first approximation it can be assigned to the C_{2h} dimer structure of the 7AI molecule.

The fact that in standard conditions the doubly-hydrogen bonded dimer is not generated obeys to solvation of the acid N-H centre (indole type hydrogen) and to the basic pyridinic centre of 7AI by the basic nitrogen, and by the acid C-H of acetonitrile, respectively. For the sake of favouring the formation of C_{2h} 7AI dimer another solvent similar in structure to acetonitrile (CH_3-CN) must be used, for example, butyronitrile ($CH_3CH_2CH_2$-CN). Therefore, with little change in the molecular characteristics of the solvent, the dipole moment is increase from 3.53 Dby for acetonitrile to 3.65 Dby for butyronitrile[20]. In addition the C-H acid component of the acetonitrile molecule is cancelled for butyronitrile, by means of balancing the electron-withdrawing inductive effect of the CN part with a longer carbon chain, thus providing a greater electron-donating effect of the ethyl group. The melting point of butyronitrile is significantly lower, and placed at 161 K. Furthermore, it is important to point out that a sample solution in butyronitrile at a temperature lower than the melting point does not undergo any cracking. Therefore, using butyronitrile allows us to investigate the spectroscopic behaviour that a sample of 7AI 0.1 M in acetonitrile would have at much lower temperatures than 218 K without any problems of cracking or lost of optical transparency. The emission spectra shown in Figures 4 and 5, and recorded from 298 to 98 K, make evident that the aggregation processes for 7AI have been favoured in butyronitrile as compared to acetonitrile; and it has been especially easier to monitor the fluorescence behaviour of the 7AI aggregate species.

Figure 4

In Figure 4 the emission spectra for a 7AI 0.1 M solution in butyronitrile are shown on excitation at 320±1 nm. At least, three distinct fluorescences are monitored. The spectra obtained between 298 and 178 K exhibit clearly one structureless

fluorescence band centred at ca. 503 nm, which at temperatures lower than 178 K overlaps with another new structured fluorescence at 479 nm. This latter becomes the dominant band below 138 K, and based on the spectral envelop, it can be C_{2h} assigned to the dimer structure of 7AI. The third fluorescence is centred at 350 nm at rt, Figure 4, and below 218 K it shifts to the red, it being centred at 366 nm at 138 K. Below 138 K, the spectra recorded within the UV spectral region can be described by several fluorescences that move their maxima to the blue, and it seems sensible to think of two fluorescences generated from monomer structures with distinct solvation.

Figure 5

The spectra shown in Figure 5 reveal three emission bands for 7AI 0.1M in butyronitrile recorded on excitation at 290±1 nm, which are the same as those mentioned above on excitation at 320 nm. However, the blue shift found for the 350 nm emission band at lower temperatures than 138 K on excitation at 320 nm could not be observed. It is especially relevant that these spectra show the structureless emission centred at 500 nm, as well as below 138 K the emission assigned to the C_{2h} 7AI dimer species can be detected. Therefore, these solutions may allow us to record the corresponding absorption spectra with the spectral changes assignable to the C_{2h} 7AI dimerization.

Figure 6 contains the absorption spectra for a 7AI 2.2x 10^{-4} M solution in butyronitrile from 298 to 128 K. From 298 to 198 K, the envelop of the first absorption band does not describe any dimerization process; as the temperature decreases the onset position is kept constant, the absorption band also increases its intensity and vibronic structure but without changing the wavelength position of the peaks. On lowering the temperature down to 158 K, less structured spectra are monitored, and down to 128 K, the spectral changes are made more evident; the spectral envelop at 128 K resembles that detected at 278 K for a 7AI 10^{-4} M solution in n-heptane (Figure 8 of Supporting Information), thus meaning that the 7AI dimerization process has started. Also, Figure 6

contains the differential spectra between those at 198 and 298 K, and those at 128 and 198 K; clearly, the first subtraction shows the increase of the monomer species with peak wavelengths at 295, 289, and 282 nm, and the second one indicates the appearance of the doubly-hydrogen bonded dimer with its characteristic peak wavelengths at 299, 292, and 284 nm.

Figure 6

The recent results obtained on a 7AI 0.1 M solution in acetonitrile[7] at rt on excitation at 320 nm show one fluorescence band assigned to the monomer species with maximum at 350 nm, and a weak fluorescence without vibronic structure at 500 nm. The authors[7] assigned the latter fluorescence to the doubly-hydrogen bonded 7AI dimer, whose analyses allow them to propose a step-wise excited state two-proton transfer reaction mechanism in which a polar intermediate is involved. K&Z propose to apply this stepwise two-proton transfer model to the DNA base pairs[7]. However, as it was mentioned above, in 1991 Moog and Maroncelli[18] reported a study of the fluorescence for a 7AI $3x10^{-5}$ M solution in acetonitrile, in which they conclude that it exhibits a fluorescence band with a peak wavelength at 350 nm and a long-wavelength tail extended to 540 nm, which they entirely ascribed to the monomer fluorescence of 7AI.

From the current work for an anhydrous 7AI 0.1 M solution in acetonitrile, the structureless emission band centred at 500 nm depends on the excitation wavelength; either by exciting at 320±1 nm or at 334±4 nm this emission band is recorded, which increases in intensity as the temperature is lowered from 298 to 218 K. However, by exciting at the maximum of the first absorption band, for example, at 290±1 nm, the long-wavelength emission band at 500 nm is absent at any of the temperatures. This behaviour indicates that the species assigned to that fluorescence does not possess a similar absorption spectral envelop to that of the monomer, for the reason that if it were close related to the monomer, for instance, it being the C_{2h} dimer, this will be excited at

any wavelength of the first absorption band, and the C_{2h} 7-azaindole dimer would emit fluorescence at 500 nm even on excitation at 290 nm as has been demonstrated for the solvents in which the 7-azaindole forms clear-cut dimers; and this is not the case for acetonitrile. The molecular species responsible for the emission with a peak maximum at 500 nm must be an aggregate different from the C_{2h} doubly-hydrogen bonded 7AI dimer or from a card-pack dimer. If it were a card-pack dimer, this will possess an absorption spectrum and a structured emission spectrum not so much shifted from the monomer species as it occurs for the 1-azacarbazole molecule[21]. For stability reasons, the band emission at 500 nm must be assigned to an aggregate possessing intermolecular hydrogen bonding interactions, as in the case of the 7AI tetramer (Scheme 1) which is the most stable structure in the crystal phase[22], see Scheme 1. Based on the excitation spectra, the new aggregate of 7AI can be selectively much easier to excite at the onset of the absorption band.

The spectral features of the emission from the doubly-hydrogen bonded C_{2h} 7AI dimer are reproduced for the emission spectrum of 7AI 0.1 M in acetonitrile at 202 K, that is, a band maximum at ca. 475 nm and two shoulders at 427 and 455 nm, but not at room temperature. Therefore, this evidence proves that there is not C_{2h} 7AI dimer in acetonitrile at 0.1 M concentration of 7AI at room temperature.

On changing from acetonitrile to butyronitrile the 7AI aggregation processes are favoured in solution, the range of temperature that can be studied is greater, and it is much easier to make evident the spectral features of the fluorescence emissions located in the visible spectral region. Under these conditions, it can be rightly stated that the long-wavelength emission band reported by Kwon and Zewail[7] is a structureless emission with peak maximum at 500 nm and most probably assignable to the tetramer species of 7AI but surely not to the C_{2h} 7AI dimer species as these authors assumed in their paper. The C_{2h} 7AI dimer species shows up at much lower temperatures than rt, such as it is demonstrated in Figure 5. On this fact, it is relevant to point out that the

emission of this C_{2h} dimer can be seen on excitation at 290±1, Figure 5, in accordance to the reasoning mentioned above.

Evidence on the C_{2h} 7AI dimerization in polar solvents leads to temperature as the control parameter. The 7AI dimerization is allowed on lowering the temperature of the solution; this key point lies in that it is feasible to generate the 7AI dimer in such a dilute 2MB solution as 10^{-6} M [23] by lowering the temperature.

The absorption spectra for 7AI $2.2x10^{-4}$ M in butyronitrile solution on lowering the temperature from 298 K to 178 K reveal the existence of the 7AI monomer species, but at 158 K some weak changes in the spectral envelop indicate the initial aggregation process, and these changes are clear-cut for the spectra monitored at 128 K. As it is shown in the spectra of Figure 6, the differential spectra between those recorded at 298 and 198 K reveal the peak maxima from the monomer species, but by subtracting the spectrum at 178 K from that one at 128 K, the resultant spectrum does not show the peaks from the monomer but those of the C_{2h} dimer. In conclusion, if there were a mixture of monomer and dimer for 7AI in acetonitrile solution at room temperature, the spectral envelop for absorption would clearly change if the relative concentration of monomer-dimer changed. In other words, it is not feasible an overlap of the monomer and dimer spectra which does not allow one to distinguish one another, as Kwon and Zewail[7] seem to assume. The absorption experiments mentioned above demonstrate that from the spectrum of the pure monomer to a mixture of monomer-dimer, the vibronic structure of the monomer disappears, and the absorption band gets red shifted; and as the dimer concentration increases a new absorption spectral envelop appears to show the vibronic structure of the dimer species, as has already been recorded in 3MP[13], 2MB[14], ClBut[15], Hep(Supporting Information), and Dec[16].

Figure 7

It is important to note that if the anhydrous condition is removed for 7AI 0.1 M in butyronitrile[10] then the structureless emission at 500 nm is not monitored, and hence,

the excitation spectra by monitoring emission light at 500 nm are an extraordinary tool to find out its ground state origin, whether it comes from monomer or dimer. Figure 7 shows the corresponding excitation spectra for 7AI 0.1 M in butyronitrile (no anhydrous) at 128 K by monitoring light at 385 and 480 nm; the absorption spectra for these monomer and dimer species are very different from one another, and therefore, for all the solvent studied they can be distinguished and spectroscopically characterized. To sum up, a) Kwon and Zewail have measured, under anhydrous conditions and assuming that two molecules were involved in the new chemical species, the formation constant not for the dimer species but for olygomer species, most probably tetramers; b) it is essential to record the corresponding excitation spectra in order to assign any fluorescence emission, and to be able to determine its ground state source, this even more necessary under large concentrations in which several aggregate species can be formed, as it happens with the 7AI case; c) from the femtosecond time-window experiments executed by Kwon and Zewail, we can only rightly state that they have studied other species than C_{2h} 7AI dimers, and therefore, their time-resolved assignments and conclusions must be revised. And d), the spectral envelop for the doubly-hydrogen-bonded dimer is clear-cut different from that of the monomer species in acetonitrile, therefore, K&Z[7] do not record changes in the first absorption band at 10^{-5} M compared with that at 0.01 M because of the doubly-hydrogen-bonded dimer species does not exist under their experimental conditions at room temperature.

For 7AI concentrated solutions, the existence of an aggregate distinct to the doubly-hydrogen bonded C_{2h} dimer species, which emits fluorescence centred at 500 nm, must foster the revision of many experiments done at large concentration of 7AI, for example 0.02 M in hydrocarbon solvents, which is frequently employed by Zewail et al. In these solutions the presence of these aggregates was invoked in 2002 in order to justify the behaviour shown by the absorption spectra of 0.02 M 7AI in hexane solution between 280 and 200 K[24], and to explain terahertz spectrum for 0.05 M 7AI in

cyclohexane solution at rt, from which Feder and Korter[25] deduce that the 7AI aggregates found are 68% dimer species and 38% tetramer species, see Scheme 1.

The evidence presented by Kwon and Zewail[7] can not be assigned to the doubly-hydrogen bonded C_{2h} 7AI dimer but to another aggregate, probably the tetramer species already reported in other studies for the solid state. The new aggregate has been generated because of the anhydrous conditions were accomplished, which at first sight is a good practice, but for the case of the 7AI molecule anhydrous polar solvents generate new aggregates, and therefore, neither change in the photophysics of the C_{2h} 7AI dimer must be invoked nor any conclusion can be extracted that may be applied to the photophysics of the DNA base pairs.

Experimental Section

7-Azaindole was obtained from Sigma-Aldrich in 99,98 % purity and it is used as supplied. All 7AI solutions contained a concentration of 0.1 or $2.2x10^{-1}$ M of the compound in the different solvents, which were purchased in the highest available purity; thus, acetonitrile are Merck Uvasol-grade (99.9 % in purity, and water content 0.01%), and butyronitrile from Aldrich (99.9 % in purity, and water content 0.009%). The sample temperature ranged from 98 to 298 K and was controlled by an Oxford DN1704 cryostat equipped with an ITC4 controller interfaced to the spectrophotometers. The cryostat was purged with dried nitrogen of 99.99% purity. UV-Vis spectra were recorded on a Cary-5 spectrophotometer. Corrected fluorescence and fluorescence excitation spectra were obtained on a calibrated Aminco Bowman AB2 spectrofluorimeter. A through experimental Section has been implemented in the Supporting Information.

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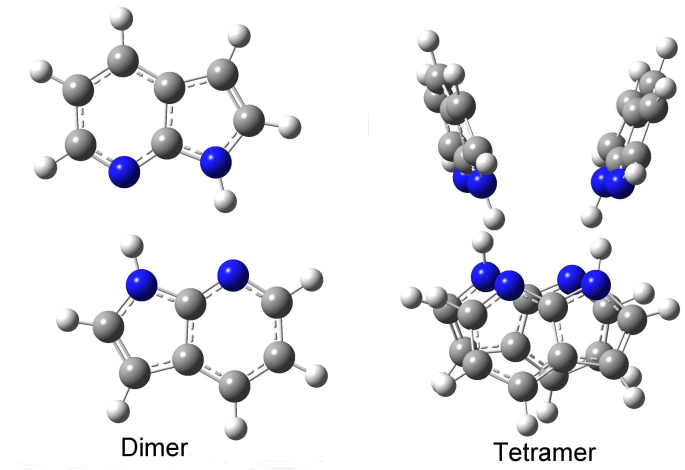
Table 1. Wavelengths(in nm) for the absorption maxima of the 7AI monomer and dimer in several solvents and for the emission maxima of he dimer.

Solvent	λ^{max}_{abs} dimer	λ^{max}_{abs} monomer	λ^{max}_{em} dimer
2-methylbutane	291.8	286.8	477
Heptane	291.8	286.8	478
3-methylpentane	293.2	287.2	480
Decalin	292.5	287.0	481
1-Chlorobutane	291.8	287.3	(a)
Acetonitrile	292(b)	287.5	475

a) non-detected, ref 15; b) see Figure 7.



Scheme 1

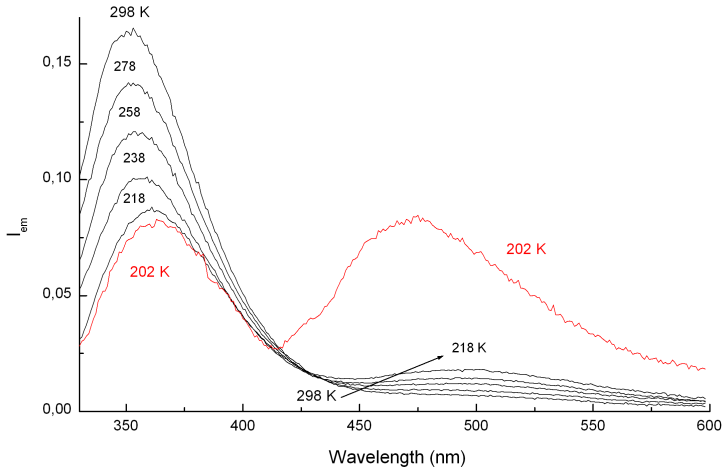


Figure 1. Emission spectra for 7AI 0.1 M in acetonitrile on excitation at 320 nm recorded from 298 K to 218 K. In red, the spectrum obtained at 202 K is plotted, obtained when the solution cracked.

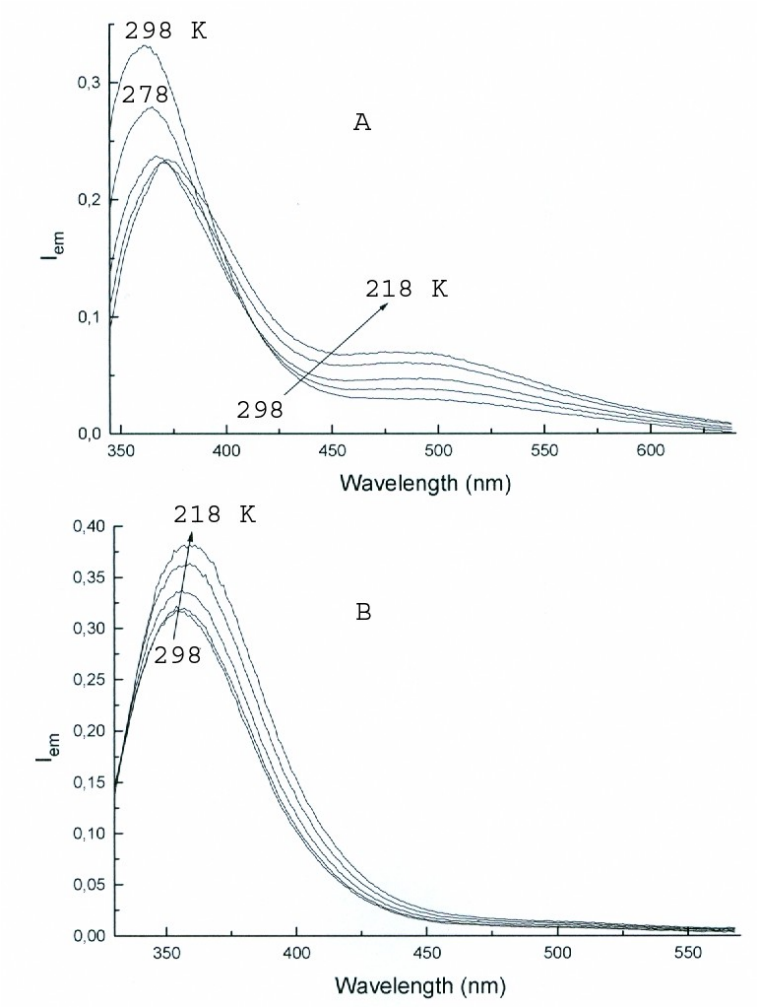


Figure 2. Emission spectra for 7AI 0.1 M in acetonitrile by exciting at: a) 334 nm and b) 290 nm, recorded from 298 to 218 K.

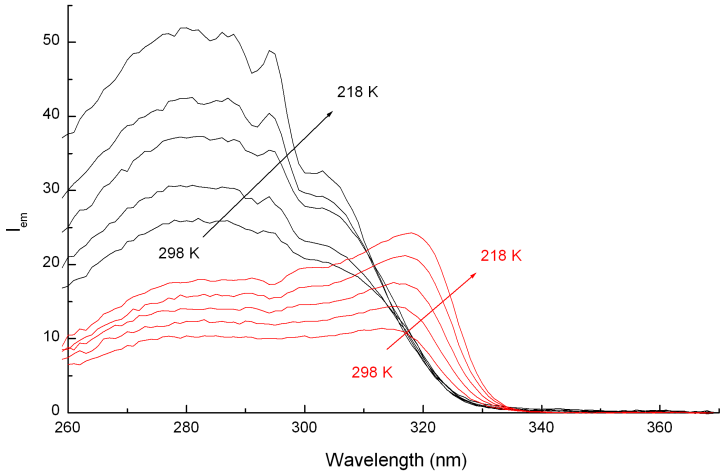


Figure 3. Excitation spectra for 7AI 0.1 M in acetonitrile by monitoring light emission at 385 nm and at 500 nm, from 298 to 218 K. The spectra obtained by monitoring at 500 nm have been multiplied by 40 for the sake of comparison with those monitored at 385 nm.

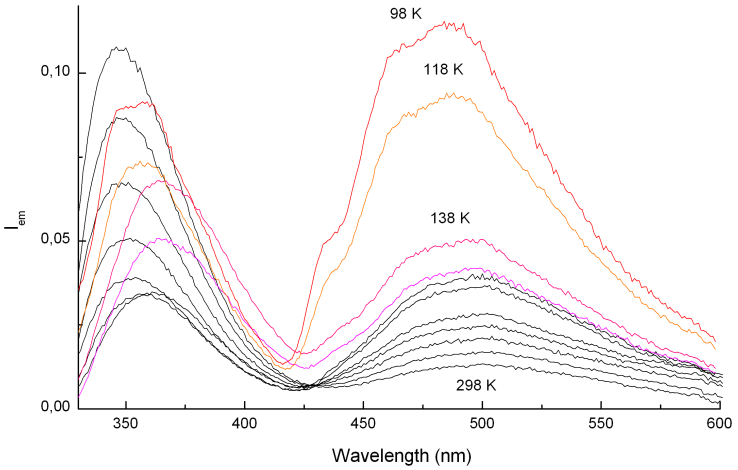


Figure 4 Emission spectra for 7AI 0.1 M in butyronitrile on excitation at 320 nm and recorded from 298 to98 K.

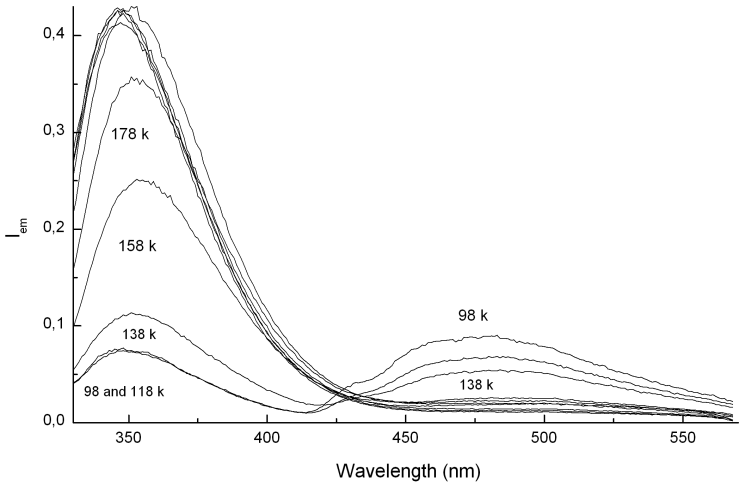


Figure 5. Emission spectra for 7AI 0.1 M in butyronitrile by exciting at 290 nm and recorded from 298 K to 98 K.

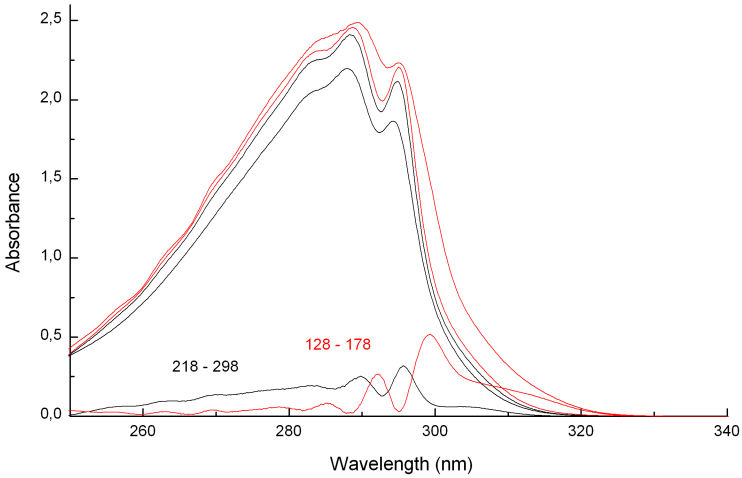


Figure 6. Absorption spectra for 7AI 2.2 x10-4 M in butyronitrile recorded from 298 to 198 K (in black), from 158 to 128 K (in red). The subtractions between those spectra recorded at 298 and 198 K (in black) and 158 and 128 K (in red) are also plotted.

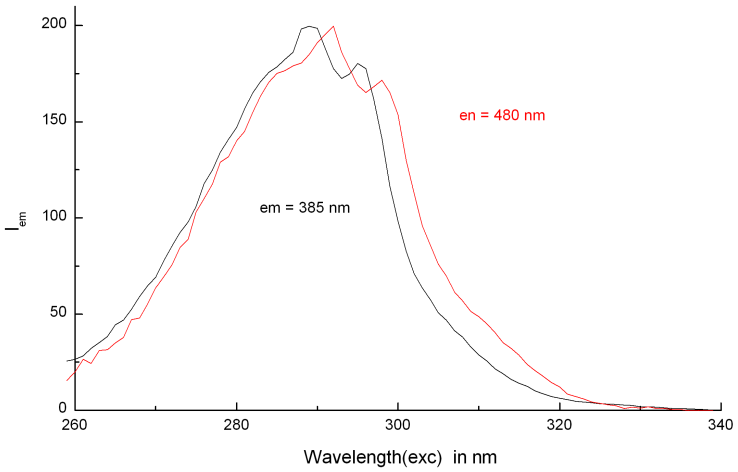


Figure 7. Excitation spectra for 7AI 0.1 M in butyronitrile (no anhydrous) by monitoring emission at 385 and 480 nm, respectively, at 128 K.

Supporting Information Available

7-Azaindole was obtained from Sigma-Aldrich in 99,98 % purity and it is used as supplied. All 7AI solutions contained a concentration of 0.1 or $2.2x10^{-1}$ M of the compound in the different solvents, which were purchased in the highest available purity; thus, acetonitrile are Merck Uvasol-grade (99.9 % in purity, and water content 0.01%), and butyronitrile from Aldrich (99.9 % in purity, and water content 0.009%). The sample temperature ranged from 98 to 298 K and was controlled by an Oxford DN1704 cryostat equipped with an ITC4 controller interfaced to the spectrophotometers. The cryostat was purged with dried nitrogen of 99.99% purity.

UV-Vis spectra were recorded on a Cary-5 spectrophotometer, using a Suprasil cell of 1 cm optical path at a variable temperature which is installed in the cryostat. All spectroscopic emission measurements were made in Suprasil cylindrical cells of 3 mm light path; as a result, the path length to this cell, which governs so-called “filtering effects” on fluorescence —a major factor with highly absorbing solutions—was less than 1 mm (the average path length ranged from 0 to 1.5 mm). All 7AI samples were excited using light from a continuous (CW) 150 W xenon lamp.

Corrected fluorescence and fluorescence excitation spectra were obtained on a calibrated Aminco Bowman AB2 spectrofluorimeter. The sensitivity factors for the emission channel of the spectrofluorimeter, which include not only those depending on the detector but also those depending on the emission monochromator and on the optical arrangement (including the channel emission), are obtained by using the correction kit FP-123 from SLM Instruments Inc. Using this kit a standard lamp is mounted in a channel at right angle from the emission channel an OL 245 M Spectral irradiance lamp (from Optronic Laboratories Inc.). This standard lamp works at constant voltage using a power supply SP-270. The light generated by the lamp is conducts into an integrating sphere, which possesses a pinhole as exit that conducts the light to the emission channel of the fluorimeter. In this way, the conversion factors obtained allow us to transform the technical spectra into the absolute spectra, which is independent of the spectrophotometer used.

The corrected excitation spectra are obtained directly in the spectrophotometer AB2, a small fraction of the light intensity for excitation is switched, by using a beam splitter, to a Hamamatsu S1336-8BQ photodiode whose photosensitivity curve *vs*. wavelength enables to find out the changes in the incident light intensity at each excitation wavelength. The ratio between the emission intensity at the wavelength monitored and the corresponding excitation intensity for each excitation wavelength provides the absolute excitation spectrum.

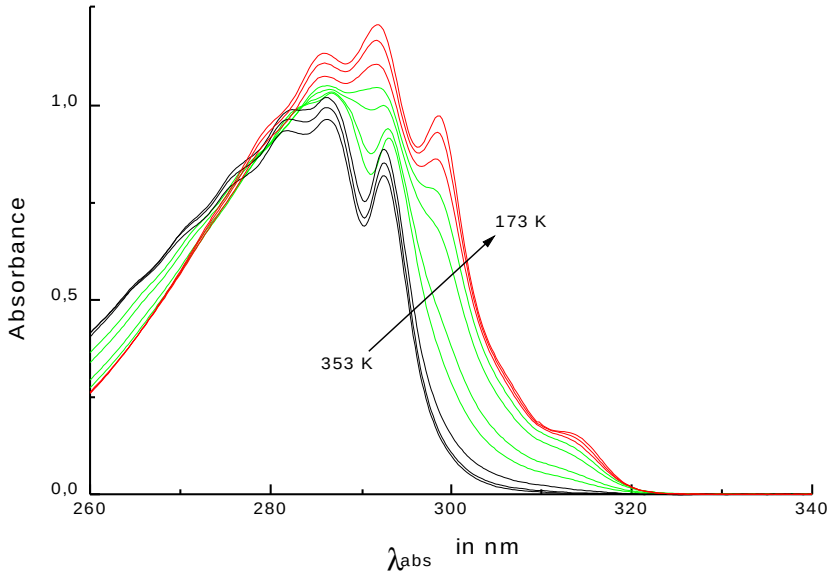


Figure 8.- Near-ultraviolet absorption spectra for a 10^{-4} M solution of 7-azaindole in n-heptane at 353, 333, 313 K (pure monomer form, in black); at 293, 273, 253, 233 K (mixture of monomer/dimer, in green); and at 213, 193 and 173 K (pure-dimer form, in red).

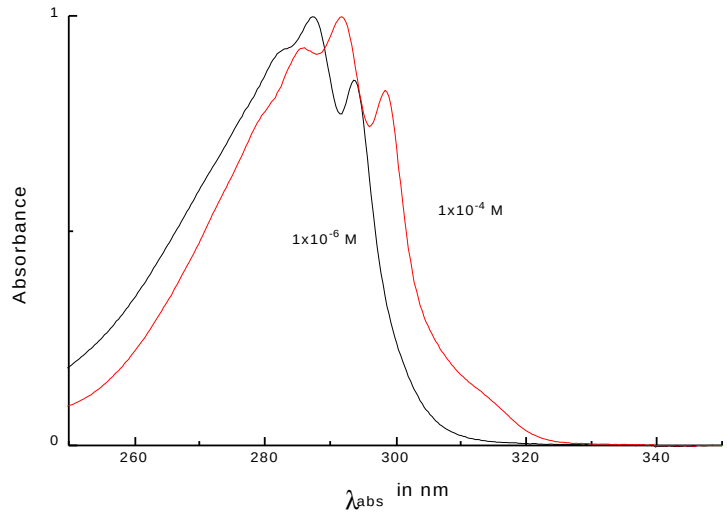


Figure 9.- Absorption spectra for 7AI monomer (in black) and dimer (in red) in Chlorobutane, normalized at the maxima. The monomer spectrum corresponds to a 10^{-6} M solution of 7AI at 298 K, in which only monomer is present; and the dimer spectrum corresponds to a 10^{-4} M solution of 7AI at 112 K, in which only dimer is present.

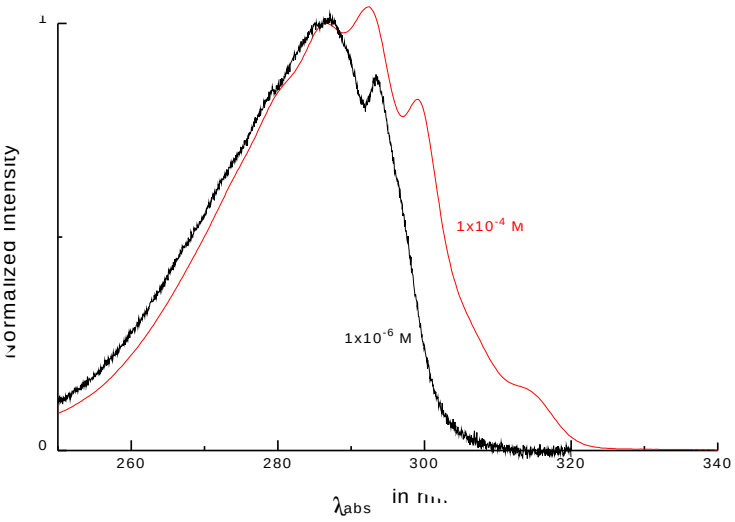


Figure 10.- Absorption spectra for 7AI monomer(in black) and dimer (in red) in decalin, normalized at the maxima. The monomer spectrum corresponds to a 10^{-6} M solution of 7AI, in which only monomer is present; and the dimer spectrum corresponds to a 10^{-4} M solution of 7AI at 112 K, in which only dimer is present.